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ORIGINAL PAPER

Influence of wine region provenance on phenolic composition, antioxidant capacity and radical scavenger activity of traditional Portuguese red grape varieties

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Abstract The introduction and spread of world-renowned varieties has caused a massive loss of indigenous grapevine varieties traditionally grown in various wine countries. In this point of view, Portugal is no exception to this situation. Thus, the aim of this study was to analyze seven traditional Portuguese red grape varieties cultivated at the same time in two wine regions of Portugal in order to determine the potential influence of these two provenances on several phenolic and antioxidant capacity parameters. Taking into account the general phenolic parameters, the highest values were found for the grape varieties cultivated in 'Dão' region (a global average value of 0.640 and 0.526 mg/g berry for total phenolic compounds and total anthocyanins, respectively). Regarding the values of total antioxidant capacity and superoxide radical scavenger activity (using SRSA method), a similar trend was observed (a global average value of 6.57 and 5.92 μmol trolox/g berry for antioxidant capacity, using ABTS and DPPH methods, respectively). However, for non-flavonoid phenols, the highest values were quantified in the samples harvested from 'Douro' region (a global average value of 0.047 mg/g berry). With this work, it was clear that the variability of the results obtained were determined by the genetic and also by environmental factors.

Keywords Anthocyanins · Phenolic compounds · Antioxidant capacity · Portuguese red grapes · Radical scavenger activity

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Introduction

Grapes have long been appreciated for their richness on phenolic compounds such as gallic acid, catechin, anthocyanins and resveratrol, and a wide variety of procyanidins [1–4]. Phenolic compounds can be classified into two groups: flavonoids and non-flavonoids. The main C_6–C_3–C_6 flavonoids in wine include conjugates of the flavonols, quercetin and myricetin; flavan-3-ols (+)-catechin and (−)-epicatechin and anthocyanins. The non-flavonoids incorporate the C_6–C_1 hydroxy-benzoic acids, and gallic and ellagic acids; the C_6–C_3 hydroxycinnmates caffeic, caftaric, and *p*-coumaric acids; and the C_6–C_2–C_6 stilbenes trans-resveratrol, *cis*-resveratrol, and *trans*-resveratrol glucoside.

Phenolic compounds, especially anthocyanins, flavonols, catechins and other flavonoids, are very important for wine quality. These compounds are responsible for most of the wine sensory characteristics, particularly the color and astringency. In addition, these compounds have also demonstrated to have a wide range of biochemical and pharmacological effects, including anticarcinogenic, antiatherogenic, anti-inflammatory, antimicrobial and antioxidant activities [5, 6].

The concentration and composition of phenolic compounds in red wine grapes differ with grape variety [1, 4,

7, 8]. However, the effects of growing conditions on grape composition, quality and yield, have to be assessed for each variety in a particular growing region. Thus, grape characteristics are influenced also by several factors such as climatic [9], geological and soil conditions, altitude of the wine region, vineyard management and crop level [10–12]. Therefore, each grape variety produced in a specific *terroir* reflects the locality in its chemical composition.

In recent years, several authors have reported on the phenolic composition and antioxidant capacity of several red and white grape varieties as well as grape berry fractions [4, 13–16]. However, over the last decades, the introduction and spread of world-renowned varieties (especially French grape varieties) has caused a massive loss of indigenous grapevine varieties traditionally grown in various grape-growing regions. The only way to prevent loss of this heritage is to study and preserve the autochthonous grape varieties, namely by studding there chemical characteristics, such as phenolic composition and antioxidant capacity.

In this point of view, Portugal is no exception to this situation. Thus, only a very few studies have been performed on the phenolic composition of the Portuguese red grape varieties, and these studies were restricted to a very small number of grape varieties from south [1, 8, 17] and north of Portugal [10, 17, 18]. In addition, it is important to note that these studies did not address the antioxidant capacity and radical scavenger activity of the autochthonous grape varieties.

So, with a view to learn more about some traditional Portuguese red grape varieties grown in two important Portuguese wine regions ('Dão' and 'Douro'), the phenolic composition (including individual anthocyanins), antioxidant capacity and radical scavenger activity of seven red grape varieties were studied. In addition, some of these Portuguese red grape varieties (especially 'Touriga Nacional' and 'Tinta Roriz') are beginning to be cultivated in other countries, such as Brazil and Australia. Thus, the present work expands the knowledge of these Portuguese red grape varieties, giving more information for a correct planning and management of the winemaking processes.

Fig. 1 Localization of the two Portuguese wine regions studied ('Douro' and 'Dão')

Materials and methods

Samples

Seven Portuguese red grape varieties (*Vitis vinifera* L.) were harvested at technological maturity with good sanitary conditions in 2011 vintage, from 6-year-old grapevines in two experimental vineyards of northeast of Portugal, one located in 'Douro' region and other in 'Dão' region (Fig. 1). Grape samples (samples of 200 berries in triplicate) were picked randomly from twenty different plants of each grape variety studied. Each grape variety sample was collected from all possible locations with difference in height and exposure to sunlight.

All grape samples were kept frozen at −20 °C until processing. The red grape varieties studied were 'Baga,' 'Castelão,' 'Mourisco Tinto,' 'Tinta Roriz,' 'Touriga Fêmea,' 'Touriga Franca' and 'Touriga Nacional.' The majority of these Portuguese red grape varieties analyzed are one of the most used in the elaboration of red wines in the two wine regions considered.

Characterization of the climatic conditions

The evolution of the climatic characteristics (average temperature and average precipitation) of the 2011 vintage from the two vineyards considered in 'Dão' and 'Douro' region are summarized in Fig. 2. In general, it was clear that during the 3 months (July, August and September) prior to sampling (at the end of September), the average temperature was higher in the 'Dão' region than in the

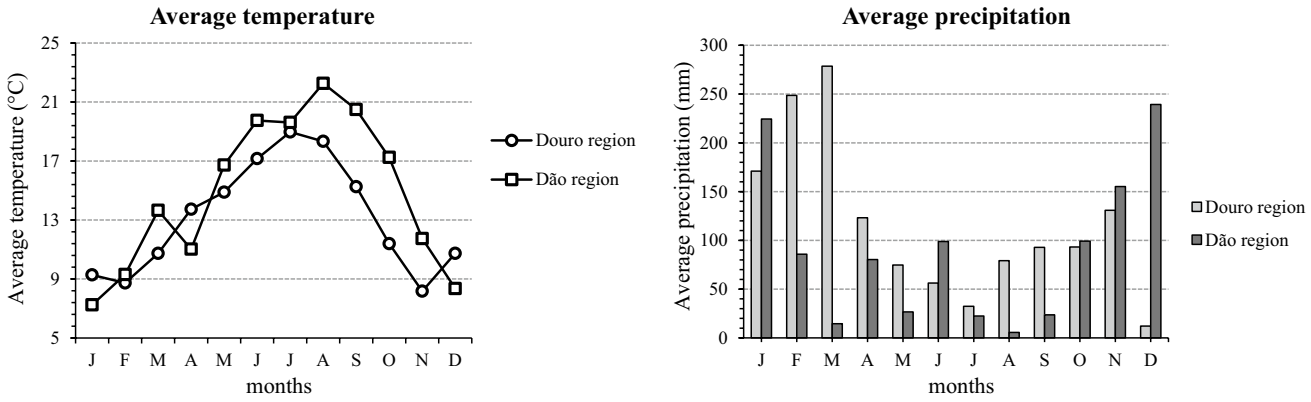

Fig. 2 Evolution of climatic characteristics (temperature and precipitation) of the 2011 vintage from the two wine regions considered

'Douro' region. In addition, average precipitation during the months of July, August and September was lower in the 'Dão' region.

Grape berries extract preparation

General physico-chemical parameters were measured directly from the must obtained after grape pressing. Phenolic parameters, antioxidant capacity and scavenger activity were determined from an extract obtained by macerating the crushed grapes at 25 °C for 24 h in a pH 3.7 buffer following the methodology proposed by Carbonneau and Champagnol [19].

General chemical and phenolic parameters

Estimated alcohol degree, pH and titratable acidity were analyzed using the analytical methods recommended by the OIV [20]. Tartaric acid was analyzed using the colorimetric determination according to Rebelein method modified by Vidal and Blouin [21]. Total anthocyanins were determined using the SO_2 bleaching method described by Ribéreau-Gayon and Stonestreet [22], while total phenolic compounds were determined by measuring absorbance at 280 nm [23]. Non-flavonoid and flavonoid phenols were determined using the method described by Kramling and Singleton [24].

Chromatographic analysis of individual anthocyanins

For the analysis of individual anthocyanins, the apparatus used was a HPLC Dionex Ultimate 3000 Chromatographic System (Sunnyvale, California, USA) equipped with a quaternary pump Model LPG-3400 A, an auto sampler Model ACC-3000, an thermostatted column compartment (adjust to 25 °C) and a multiple Wavelength Detector MWD-300. The column (250 × 4.6 mm, particle size 5 μm) was a C_{18} Acclaim® 120 (Dionex, Sunnyvale, California, USA) protected by a guard column of the same material. The solvents were (A) 40 % formic acid, (B) pure acetonitrile and (C) bidistilled water. The monomeric anthocyanins were analyzed by HPLC using the method described by Dallas and Laureano [25]. Thus, initial conditions were 25 % A, 10 % B and 65 % C, followed by a linear gradient from 10 to 30 % B, and 65 to 45 % C for 40 min, with a flow rate of 0.7 mL/min. The injection volume was 40 μL. The detection was made at 520 nm, and a Chromeleon software program version 6.8 (Sunnyvale, California, USA) was used. The quantification of the monomeric anthocyanins was made by mean of calibration curve obtained with standard solutions of malvidin-3-glucoside chloride (>95 % purity) from Extra-synthese (Genay, France). The chromatographic peaks of anthocyanins were identified according to reference data previously described by Dallas and Laureano [25]. All analyses were done in triplicate.

Although only low levels of some of them were present, 12 individual anthocyanins were detected, as shown on the chromatogram of a red grape extract (Fig. 3).

Antioxidant capacity

ABTS and DPPH methods

ABTS method is based on discoloration that occurs when the radical cation $ABTS^{\cdot+}$ is reduced to ABTS (2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) [26]. The radical was generated by reaction of a 7 mM solution of ABTS in water with 2.45 mM potassium persulphate (1:1). The assay was made up with 980 μL of $ABTS^{+}$ solutions and 20 μL of the sample (at a dilution of 1:50 in water). The reaction takes place in darkness at room temperature.

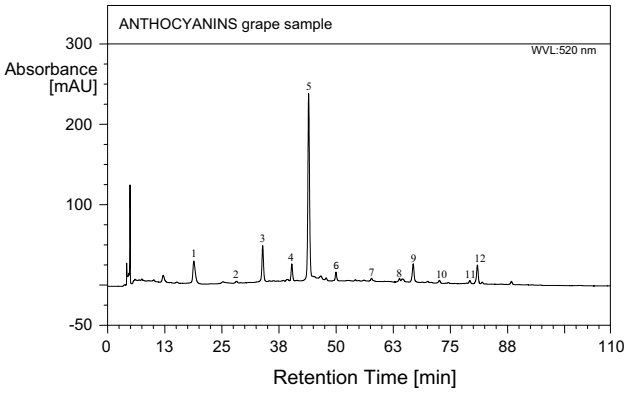

Fig. 3 Chromatogram of individual anthocyanins from a red grape sample. The peaks correspond to: *1* delphinidin-3-glucoside, *2* cyanidin-3-glucoside, *3* petunidin-3-glucoside, *4* peonidin-3-glucoside, *5* malvidin-3-glucoside, *6* cyanidin-3-acetylglucoside, *7* petunidin-3-acetylglucoside, *8* peonidin-3-acetylglucoside, *9* malvidin-3-acetylglucoside, *10* petunidin-3-*p*-coumaroyl glucoside, *11* peonidin-3-*p*-coumaroyl glucoside, *12* malvidin-3-*p*-coumaroyl glucoside

Absorbance measurements at 734 nm were made after 15 min of reaction time.

The procedure used to determine antioxidant activity using DPPH method is described by Brand-Williams et al. [27]. Briefly, 0.1 mL of different sample concentrations was added to 3.9 mL of 2.2-diphenyl-1-pirylhydrazyl (DPPH) methanolic solution (25 mg/L). The DPPH solution was prepared daily and protected from the light. Absorbance at 515 nm was measured after 30 min of reaction at 20 °C. The reaction was carried out under shaking in closed Eppendorf tubes at 20 °C. Methanol was used as a blank reference.

The antioxidant capacity results obtained from ABTS and DPPH methods were expressed as Trolox equivalents (TEAC mM), using the relevant calibration curve. All analyses were performed in triplicate.

Radical scavenger activity

Radical scavenger activities toward hydroxyl radical (HRSA) and superoxide radical (SRSA) were measured, because they are the two of the most biological active reactive oxygen species (ROS).

Hydroxyl radical-scavenging activity (HRSA)

Desoxyribose (2-desoxy-D-ribose) decays when exposed to hydroxyl radicals generated by the Fenton reaction [28]. The hydroxyl radicals (HO·) were generated through the following system: 10 μL of $FeCl_3$ (0.1 mmol/L), 10 μL of ascorbic acid (0.1 mmol/L), 10 μL of H_2O_2 (1 mmol/L) and 10 μL of EDTA (0.1 mmol/L). Samples (15 μL at a dilution of 1:50 in distilled water) were incubated at 37 °C for 1 h, with 20 μL of desoxyribose (1 mmol/L final concentration) in the presence of $FeCl_3$, ascorbic acid, H_2O_2 and EDTA. 1.5 mL of trichloroacetic acid (28 %, w/v) and 1 mL of thiobarbituric acid (1 %, w/v, 0.05 mol/L NaOH) were added to 1 mL of the sample under incubation and held for 15 min at 100 °C, after which it was left to cool to room temperature. The malondialdehyde formed from the decay of desoxyribose was evaluated in reaction with thiobarbituric acid and measured at 532 nm. The final results were expressed as inhibition percent in relation to a control test (without the sample).

Superoxide radical-scavenging activity (SRSA)

The superoxide radical reacts with 4-nitroblue tetrazolium chloride to generate a colored compound with absorbance to 560 nm [29]. The antioxidant-scavenging superoxide radical values are associated with the coloration formed during the reaction process. The reactive solution was made with 50 μL of nicotinamide (77 μmol/L), 50 μL of 4-nitroblue tetrazolium chloride (50 μmol/L) and 5 μL of phenazine methosulfate (3.3 μmol/L final concentration) in a medium of 16 mmol/L Tris–HCl, pH 8.0 and 10 μL of the sample. The results were expressed as a percentage of inhibition in relation to a control test (without the sample). All measurements were performed in triplicate.

Statistical analysis

The data are presented as mean ± standard deviation. To determine whether there is a statistically significant difference between the data obtained for the diverse phenolic compounds, antioxidant capacity and scavenger activity in the different grape varieties, an analysis of variance (ANOVA, one-way) and comparison of treatment means were carried out using Statistica 7 Software (StatSoft, Tulsa, USA). Scheffler's test was applied to the data as comparison test to determine when samples are significantly different after ANOVA ($p < 0.05$). Principal component analysis (PCA) was used to analyze the data and study the relations between the grape varieties and their physico-chemical composition, phenolic content, antioxidant capacity or scavenger activity.

Results and discussion

General physico-chemical and phenolic composition of grapes

Table 1 shows the results of the general physico-chemical composition of the different traditional Portuguese red

Table 1 General physico-chemical composition of grape must from the grape varieties studied at technological maturity

Cultivar/Region	Grape berry weight[1] (g)	Must volume[2] (mL)	Estimated alcohol degree (% v/v)	pH	Titratable acidity (g/L tartaric acid)	Tartaric acid (g/kg grapes)
Baga/Dão	234 ± 2^{a}	97 ± 1^{a}	12.44 ± 0.01^{e}	2.76 ± 0.03^{b}	6.38 ± 0.03^{h}	1.2 ± 0.1^{c}
Baga/Douro	305 ± 3^{b}	191 ± 3^{b}	11.07 ± 0.01^{a}	2.89 ± 0.02^{c}	6.40 ± 0.02^{h}	1.6 ± 0.0^{f}
Castelão/Dão	275 ± 1^{c}	130 ± 2^{c}	13.61 ± 0.06^{h}	2.98 ± 0.02^{c}	4.50 ± 0.01^{d}	1.0 ± 0.0^{a}
Castelão/Douro	303 ± 3^{b}	137 ± 1^{c}	13.06 ± 0.02^{g}	2.92 ± 0.01^{c}	7.20 ± 0.04^{j}	0.9 ± 0.0^{a}
Mourisco Tinto/Dão	290 ± 5^{d}	160 ± 4^{d}	12.98 ± 0.02^{g}	2.95 ± 0.04^{c}	6.38 ± 0.03^{h}	1.0 ± 0.0^{a}
Mourisco Tinto/Douro	356 ± 3^{e}	194 ± 3^{b}	11.87 ± 0.01^{c}	2.89 ± 0.00^{c}	6.80 ± 0.01^{i}	1.3 ± 0.0^{d}
Tinta Roriz/Dão	370 ± 4^{e}	191 ± 5^{b}	13.62 ± 0.04^{i}	3.30 ± 0.02^{f}	3.26 ± 0.01^{a}	1.1 ± 0.0^{b}
Tinta Roriz/Douro	410 ± 6^{f}	223 ± 2^{e}	14.08 ± 0.01^{j}	3.11 ± 0.01^{e}	4.80 ± 0.02^{e}	1.8 ± 0.0^{f}
Touriga Fêmea/Dão	218 ± 2^{g}	119 ± 3^{f}	12.02 ± 0.06^{d}	2.68 ± 0.00^{a}	6.38 ± 0.01^{h}	1.2 ± 0.1^{d}
Touriga Fêmea/Douro	267 ± 3^{c}	129 ± 1^{c}	12.01 ± 0.01^{d}	3.28 ± 0.01^{f}	7.10 ± 0.01^{j}	1.5 ± 0.0^{e}
Touriga Franca/Dão	258 ± 4^{h}	128 ± 7^{c}	12.50 ± 0.07^{e}	3.30 ± 0.02^{f}	4.09 ± 0.02^{c}	0.9 ± 0.0^{a}
Touriga Franca/Douro	472 ± 6^{i}	217 ± 5^{e}	11.31 ± 0.02^{b}	2.96 ± 0.03^{d}	5.20 ± 0.01^{f}	1.0 ± 0.0^{a}
Touriga Nacional/Dão	254 ± 1^{h}	142 ± 4^{c}	12.91 ± 0.04^{f}	3.19 ± 0.01^{e}	3.78 ± 0.03^{b}	0.8 ± 0.1^{a}
Touriga Nacional/Douro	314 ± 2^{b}	136 ± 6^{c}	13.97 ± 0.01^{j}	2.81 ± 0.01^{b}	6.00 ± 0.01^{g}	1.6 ± 0.2^{e}
Global average value/Dão	271 ± 2.7^{A}	138 ± 3.7^{A}	12.80 ± 0.4^{A}	3.02 ± 0.2^{A}	4.96 ± 0.2^{A}	1.0 ± 0.2^{A}
Global average value/Douro	347 ± 3.7^{B}	175 ± 3.0^{B}	12.40 ± 1.4^{A}	2.98 ± 0.2^{A}	6.21 ± 0.01^{B}	1.4 ± 0.3^{B}

[1] Grape berry weight from 200 berries

[2] Must volume extracted from 200 berries

All data expresses the average of three replicates ± standard deviation

Different letters indicates the existence of statistical differences for each column ($p < 0.05$)

grape varieties harvested at technological maturity from the two vineyards considered.

Making an overall analysis of the results obtained from the different grape varieties studied, it was observed that the samples collected in the vineyard located in the 'Douro' region showed a significantly higher global average value for grape berry weight (347 g), must volume (175 mL), titratable acidity (6.21 g/L tartaric acid) and tartaric acid content (1.4 g/kg grapes). Samples harvested in the vineyard located in the 'Dão' region showed a slightly higher global average value for estimated alcohol degree (12.80 %) and pH value (pH 3.02). The results obtained for the grapes from the 'Dão' region may have been favored by the higher average temperature during the months of June, July, August and September of this region (Fig. 1) compared to that observed in the 'Douro' region. Thus, these conditions may have contributed to the accumulation of sugars and to higher levels of organic acid (e.g., tartaric acid) degradation during grape maturation, compared to that observed in grape samples from the vineyard located in the 'Douro' region. On the other hand, precipitation was higher in the 'Douro' region, during the months corresponding to the end of grapes maturation (in particular during September with an average precipitation of 92.8 mm in 'Douro' region and 23.6 mm in 'Dão' region). This fact may have contributed to the achievement of significantly higher global average value for grape berry weight and further delay in the grapes maturation proceeding.

Concerning to the individual results for each red grape variety, 'Tinta Roriz' and 'Touriga Franca' harvested from the vineyard located in 'Douro' region showed the significantly highest values of grape berry weight (410 and 472 g, respectively) and must volume (223 and 217 mL, respectively). On the other hand, 'Baga' and 'Touriga Fêmea' from 'Dão' region vineyard showed the significantly lowest grape berry weight (234 and 218 g, respectively) and must volume (97 and 119 mL, respectively).

With regard to the estimated alcohol degree from grape must, the values ranged from 11.07 % (v/v) ('Baga' variety from the vineyard located in 'Douro' region) to 14.08 % (v/v) ('Tinta Roriz' variety from the vineyard located in 'Douro' region). All grape varieties showed higher estimated alcohol degree when cultivate in 'Dão' region vineyard than in 'Douro' region vineyard, except for 'Tinta Roriz' and 'Touriga Nacional' grape varieties.

'Castelão' and 'Touriga Fêmea' exhibited the highest titratable acidity (7.20 and 7.10 g/L tartaric acid, respectively) when cultivated in 'Douro' region vineyard. It is important to consider that the high level of acidity determined was very dependent of the grape vineyard region, because the highest titratable acidity values were obtained

Table 2 General phenolic composition of the grape varieties studied at technological maturity

Cultivar/Region	Total phenol index	Total phenolic compounds (mg/g berry)	Total anthocyanins (mg/g berry)	Non-flavonoid compounds (mg/g berry)	Flavonoid compounds (mg/g berry)
Baga/Dão	67 ± 1^{d}	0.519 ± 0.001^{fg}	0.386 ± 0.031^{d}	0.049 ± 0.001^{i}	0.469 ± 0.001^{fg}
Baga/Douro	58 ± 0^{b}	0.573 ± 0.003^{c}	0.366 ± 0.009^{d}	0.042 ± 0.000^{e}	0.531 ± 0.003^{c}
Castelão/Dão	91 ± 0^{g}	0.528 ± 0.013^{d}	0.395 ± 0.063^{d}	0.031 ± 0.000^{c}	0.497 ± 0.013^{de}
Castelão/Douro	63 ± 1^{c}	0.351 ± 0.001^{b}	0.296 ± 0.03^{c}	0.032 ± 0.000^{c}	0.319 ± 0.000^{b}
Mourisco Tinto/Dão	63 ± 0^{c}	0.626 ± 0.007^{d}	0.515 ± 0.062^{f}	0.049 ± 0.001^{f}	0.577 ± 0.008^{cd}
Mourisco Tinto/Douro	48 ± 0^{a}	0.464 ± 0.003^{b}	0.114 ± 0.005^{a}	0.058 ± 0.000^{h}	0.406 ± 0.003^{b}
Tinta Roriz/Dão	85 ± 1^{f}	0.646 ± 0.004^{fg}	0.811 ± 0.002^{h}	0.047 ± 0.000^{f}	0.599 ± 0.004^{fg}
Tinta Roriz/Douro	83 ± 1^{f}	0.685 ± 0.020^{g}	0.658 ± 0.015^{gh}	0.051 ± 0.001^{g}	0.634 ± 0.021^{gh}
Touriga Fêmea/Dão	66 ± 0^{d}	0.655 ± 0.003^{ef}	0.470 ± 0.001^{e}	0.027 ± 0.000^{b}	0.628 ± 0.002^{fg}
Touriga Fêmea/Douro	49 ± 0^{a}	0.558 ± 0.000^{de}	0.213 ± 0.014^{b}	0.038 ± 0.000^{de}	0.521 ± 0.000^{ef}
Touriga Franca/Dão	76 ± 1^{e}	0.717 ± 0.007^{h}	0.498 ± 0.011^{ef}	0.019 ± 0.000^{a}	0.698 ± 0.007^{j}
Touriga Franca//Douro	58 ± 1^{b}	0.568 ± 0.003^{fg}	0.430 ± 0.011^{e}	0.045 ± 0.001^{g}	0.523 ± 0.004^{fg}
Touriga Nacional/Dão	72 ± 3^{de}	0.790 ± 0.007^{a}	0.594 ± 0.026^{fg}	0.077 ± 0.000^{k}	0.713 ± 0.007^{a}
Touriga Nacional/Douro	95 ± 0^{g}	0.274 ± 0.000^{h}	0.762 ± 0.021^{gh}	0.062 ± 0.000^{j}	0.211 ± 0.000^{h}
Global average value/Dão	74 ± 10.4^{A}	0.640 ± 0.093^{A}	0.526 ± 0.135^{A}	0.043 ± 0.019^{A}	0.597 ± 0.089^{A}
Global average value/Douro	65 ± 17.0^{B}	0.496 ± 0.137^{B}	0.403 ± 0.227^{B}	0.047 ± 0.010^{B}	0.449 ± 0.140^{B}

All data expresses the average of three replicates ± standard deviation

Different letters indicates the existence of statistical differences for each column ($p < 0.05$)

in the grape varieties from the vineyard located in the 'Douro' region.

General phenolic composition of the Portuguese red grape varieties studied for the both wine regions considered is shown in Table 2. Thus, making an overall analysis of the results, all general phenolic parameters analyzed (except for non-flavonoid compounds) showed significantly higher global averages values in the grape varieties harvested in the vineyard located in 'Dão' region. For example, for total phenolic compounds, grape varieties harvested in the vineyard located in 'Dão' region exhibited a global average value of 0.640 mg/g of berry, while the same grape varieties harvested in the vineyard located in 'Douro' region exhibited a global average value of 0.496 mg/g of berry. Similar trend was quantified for total anthocyanins (0.526 and 0.403 mg/g of berry for grape varieties harvested in the vineyard located in 'Dão' and 'Douro' region, respectively).

The low precipitation in 2011, in 'Dão' region during August and September (5.6 and 23.6 mm, respectively) compared to the precipitation in 'Douro' region for the same months (79.2 and 92.8 mm, respectively), could probably contribute to an activation of the flavonoid pathway responsible for tannin and anthocyanin biosynthesis which occurs during the grape maturation [30]. This observation may help to explain the higher phenolic content quantified in the grape varieties harvested in the vineyard localized in 'Dão' region. Several investigators have also mentioned the impact of climatic conditions such as average temperatures as factors that have an important impact in grape polyphenol accumulation [31, 32]. Thus, in the 'Dão' region, the average temperatures in August and September were 22.2 °C and 20.4 °C, respectively, while in 'Douro' region, the average temperatures in August and September were much more lower (18.3 and 15.3 °C, respectively). Thus, all of these conditions contributed to higher phenolic compound accumulation in grapes harvested in the vineyard located in 'Dão' region.

Regarding individual results of each grape cultivar, total phenolic compounds ranged from 0.274 ('Touriga Nacional' from 'Douro' region vineyard) to 0.790 mg/g of berry ('Touriga Nacional' from 'Dão' region vineyard). Thus, taking into account this parameter for each grape variety and region, the highest total phenolic compounds was found for the grape varieties from the vineyard localized in 'Dão' region, except for 'Baga' and 'Tinta Roriz' where the highest total phenolic compounds were quantified in the grapes from the vineyard localized in 'Douro' region (0.573 and 0.685 mg/g of berry, respectively). Recently Costa et al. [4] reported similar values of total phenolic compounds from 18 different autochthonous and non-autochthonous grape varieties cultivated in 'Douro' region. However, Jordão et al. [2] quantified lower levels of total phenolic compounds in 'Touriga Franca' and 'Castelão' grape varieties harvested from a vineyard localized in south of Portugal.

Concerning the concentration of total anthocyanins, the values ranged from 0.114 ('Mourisco Tinto' from 'Douro' region vineyard) to 0.811 mg/g of berry ('Tinta Roriz' from 'Dão' region vineyard). It was also clear that grape varieties harvested from the vineyard localized in 'Dão' region showed higher total anthocyanin content than the same grape varieties harvested in the vineyard localized in 'Douro' region. However, 'Touriga Nacional' was an exception, presenting higher values for total anthocyanins on the samples coming from the vineyard localized in 'Douro' region (0.762 and 0.594 mg/g of berry for 'Douro' and 'Dão' region vineyard, respectively).

The seven grape varieties studied in this work from the two wine regions considered presented a total anthocyanin content similar to that of most of the international grape varieties grown and used for winemaking all over the world (such as Cabernet Sauvignon, Syrah and Merlot) [33, 34]. In addition, Guerrero et al. [35] analyzed the quantitative differences in total anthocyanins from 5 different red grape varieties from Andalusia region (Spain) and found a higher range for total anthocyanin content (from 0.906 to 2.64 mg/g of berry).

It is important to note that the experience has shown that highly colored grapes and total anthocyanin content do not necessarily produce high colored wines; any differences are probably related to the easiness of anthocyanins extraction from grape skins into grape must [36].

Finally, for non-flavonoid compounds, an opposite tendency was found, i.e., the highest content of non-flavonoid compounds was associated with grape samples from the vineyard localized in 'Douro' region (global average value of 0.047 mg/g of berry) than from 'Dão' region (global average value of 0.043 mg/g of berry). However, for 'Baga' and 'Touriga Nacional,' the highest non-flavonoid compounds were quantified in samples harvested in the vineyard localized in 'Dão' region (0.049 and 0.077 mg/g of berry, respectively).

Considering these results, probably there is a degree of adaptation of the majority of grape varieties studied to climate and soil conditions from 'Douro' region with respect to the biosynthesis and accumulation of non-flavonoid compounds, that include stilbenoids (such as resveratrol) and phenolic acids (such as benzoic, caffeic, caftaric and cinnamic acids). In early studies, a negative relationship was reported between resveratrol content and fruit maturity, that is resveratrol declined during fruit maturity, and *trans*-resveratrol had a negative correlation with both anthocyanin and sugar accumulation of grape skin and grape juice [37, 38]. Thus, in this way, it is important to remember that the grape samples from 'Douro' region vineyard had in general less total anthocyanin content and estimated alcohol degree (see Table 1). Finally, it is important to consider that according to Versari et al. [39], the decreased ability of grape skin to synthesize stilbenes at ripening may be due to an increase of chalcone synthase activity, an enzyme that utilizes the same subtract that is related with anthocyanin accumulation.

Individual anthocyanin composition

Taking into account the results shown in Table 3, in general the global content of each individual anthocyanins was significantly higher in the grape varieties harvested in 'Dão' region vineyard. Thus, the global content of the individual anthocyanins for the different grape varieties studied ranged from 0.003 to 0.226 mg/g of berry and from 0.001 to 0.208 mg/g of berry, respectively, for the grape samples from 'Dão' and 'Douro' region. Such a high level of individual anthocyanins in grape samples from 'Dão' region vineyard was mainly attributed to its higher abundance in malvidin derivatives (average global value of 0.226, 0.036 and 0.032 mg/g of berry, for malvidin-3-glucoside, malvidin-3-acetylglucoside and malvidin-3-*p*-coumaroyl glucoside, respectively) and delphinidin-3-glucoside (global average value of 0.051 mg/g of berry).

In general, although the grape varieties grown in 'Dão' region vineyard have shown higher levels of individual anthocyanins, 'Baga' and 'Touriga Nacional' varieties were an exception to this trend, presenting a higher concentration for the majority of individual anthocyanins quantified from the grape samples harvested in vineyard located in 'Douro' region.

The results presented previously suggested that the individual anthocyanins accumulation in the Portuguese red grape varieties studied are strongly affected by 'terroir' factors which confirm the results obtained for general phenolic composition (see Table 2) and by other authors with other grape varieties and wine regions [10]. Thus, 'terroir' factor is believed to participate in the regulation of phenolic compound biosynthesis in grape berries, in this case for anthocyanins, ultimately determining the geographical features of wines.

For all grape varieties studied, malvidin-3-glucoside was the major individual anthocyanin quantified (ranging from 0.058 to 0.370 mg/g of berry). These results are in agreement with previous findings quantified in other grape varieties [1, 4, 7, 17, 36]. According to Ribéreau-Gayon et al. [40], malvidin derivative forms are stable molecules and their presence gives stability to the wine during winemaking process, because these compounds are relatively resistant to oxidant process. In opposite, petunidin-3-acetylglucoside, cyanidin-3-acetylglucoside and peonidin-3-acetylglucoside were less abundant individual anthocyanins (ranging from 0.001 to 0.021, from 0.002 to 0.018 and from 0.002 to 0.005 mg/g of berry, respectively). In addition, these three anthocyanins were not detected in

Table 3 Individual anthocyanins quantified in the grape berry of the varieties studied at technological maturity

Cultivar/region	Delp gluc	Cyan gluc	Petun gluc	Peon gluc	Malv gluc	Cyanacet-gluc	Petunacet-gluc	Peonacet-gluc	Malvacet-gluc	Petuncoum-gluc	Peoncoum-gluc	Malvcoum-gluc
Baga/Dão	0.020 ± 0.001^{bc}	n.d.	0.029 ± 0.001^{bc}	0.031 ± 0.001^{b}	0.115 ± 0.006^{b}	n.d.	0.008 ± 0.000^{c}	0.005 ± 0.001^{b}	0.010 ± 0.000^{b}	0.020 ± 0.004^{d}	0.004 ± 0.001^{a}	0.014 ± 0.004^{c}
Baga/Douro	0.011 ± 0.001^{ab}	0.004 ± 0.001^{cd}	0.025 ± 0.000^{bc}	0.071 ± 0.003^{e}	0.227 ± 0.007^{c}	n.d.	n.d.	n.d.	0.004 ± 0.001^{a}	n.d.	n.d.	0.002 ± 0.001^{a}
Castelão/Dão	0.021 ± 0.001^{bc}	0.003 ± 0.000^{cd}	0.023 ± 0.001^{bc}	0.042 ± 0.001^{c}	0.120 ± 0.002^{b}	0.004 ± 0.000^{b}	0.007 ± 0.000^{c}	n.d.	0.015 ± 0.002^{b}	0.004 ± 0.000^{a}	0.014 ± 0.001^{c}	0.026 ± 0.005^{d}
Castelão/Douro	0.019 ± 0.005^{bc}	0.006 ± 0.000^{e}	0.028 ± 0.001^{bc}	0.056 ± 0.012^{d}	0.118 ± 0.004^{b}	n.d.	n.d.	n.d.	0.009 ± 0.001^{b}	0.005 ± 0.000^{a}	0.003 ± 0.001^{a}	0.016 ± 0.003^{c}
Mourisco Tinto/Dão	0.017 ± 0.010^{bc}	0.003 ± 0.000^{cd}	0.038 ± 0.001^{c}	0.048 ± 0.000^{c}	0.272 ± 0.003^{d}	0.002 ± 0.000^{a}	0.005 ± 0.001^{b}	n.d.	0.062 ± 0.001^{e}	0.004 ± 0.000^{a}	0.006 ± 0.001^{b}	0.036 ± 0.003^{e}
Mourisco Tinto/Douro	0.011 ± 0.001^{ab}	n.d.	0.001 ± 0.000^{a}	0.014 ± 0.001^{a}	0.058 ± 0.001^{a}	n.d.	n.d.	n.d.	0.005 ± 0.001^{a}	n.d.	n.d.	0.005 ± 0.001^{b}
Tinta Roriz/Dão	0.204 ± 0.002^{f}	0.011 ± 0.000^{f}	0.055 ± 0.000^{d}	0.033 ± 0.000^{b}	0.348 ± 0.002^{f}	n.d.	n.d.	n.d.	0.024 ± 0.001^{c}	0.004 ± 0.000^{a}	0.004 ± 0.001^{a}	0.021 ± 0.001^{d}
Tinta Roriz/Douro	0.096 ± 0.002^{e}	0.018 ± 0.000^{h}	0.074 ± 0.006^{e}	0.056 ± 0.000^{d}	0.305 ± 0.002^{e}	n.d.	0.001 ± 0.000^{a}	0.005 ± 0.001^{b}	0.026 ± 0.001^{c}	0.011 ± 0.001^{c}	0.004 ± 0.001^{a}	0.034 ± 0.001^{e}
Touriga Fêmea/Dão	0.043 ± 0.001^{d}	0.002 ± 0.000^{bc}	0.075 ± 0.006^{e}	0.030 ± 0.000^{b}	0.145 ± 0.007^{b}	0.018 ± 0.002^{c}	0.021 ± 0.001^{d}	0.002 ± 0.001^{a}	0.075 ± 0.007^{e}	0.003 ± 0.001^{a}	0.002 ± 0.000^{a}	0.025 ± 0.001^{d}
Touriga Fêmea/Douro	0.011 ± 0.001^{ab}	0.001 ± 0.000^{a}	0.015 ± 0.002^{ab}	0.009 ± 0.000^{a}	0.122 ± 0.001^{b}	n.d.	n.d.	n.d.	0.011 ± 0.001^{b}	0.009 ± 0.001^{b}	n.d.	0.024 ± 0.005^{d}
Touriga Franca/Dão	0.017 ± 0.005^{bc}	0.002 ± 0.003^{bc}	0.026 ± 0.000^{bc}	0.044 ± 0.001^{c}	0.273 ± 0.006^{d}	n.d.	0.002 ± 0.000^{a}	n.d.	0.054 ± 0.001^{d}	0.008 ± 0.001^{b}	0.005 ± 0.001^{a}	0.040 ± 0.002^{f}
Touriga Franca/Douro	0.002 ± 0.001^{a}	0.014 ± 0.001^{g}	0.017 ± 0.001^{ab}	0.085 ± 0.001^{f}	0.257 ± 0.002^{c}	n.d.	n.d.	n.d.	0.016 ± 0.001^{b}	0.006 ± 0.001^{b}	n.d.	0.012 ± 0.002^{c}
Touriga Nacional/Dão	0.034 ± 0.000^{cd}	0.004 ± 0.000^{de}	0.056 ± 0.001^{d}	0.067 ± 0.001^{d}	0.311 ± 0.013^{e}	0.002 ± 0.000^{a}	0.005 ± 0.001^{b}	n.d.	0.013 ± 0.004^{b}	0.004 ± 0.000^{a}	0.006 ± 0.001^{b}	0.065 ± 0.006^{g}
Touriga Nacional/Douro	0.022 ± 0.000^{bc}	0.002 ± 0.000^{bc}	0.028 ± 0.001^{bc}	0.043 ± 0.001^{c}	0.370 ± 0.033^{f}	n.d.	0.001 ± 0.000^{a}	n.d.	0.066 ± 0.001^{e}	0.006 ± 0.001^{b}	0.004 ± 0.000^{a}	0.041 ± 0.004^{f}
Global average value/Dão	0.051 ± 0.036^{A}	0.004 ± 0.002^{A}	0.043 ± 0.019^{A}	0.042 ± 0.012^{A}	0.226 ± 0.093^{A}	0.006 ± 0.001^{A}	0.008 ± 0.003^{A}	0.003 ± 0.001^{A}	0.036 ± 0.016^{A}	0.007 ± 0.003^{A}	0.006 ± 0.002^{A}	0.032 ± 0.016^{A}
Global average value/Douro	0.024 ± 0.015^{B}	0.006 ± 0.003^{B}	0.027 ± 0.011^{B}	0.048 ± 0.027^{A}	0.208 ± 0.109^{A}	n.d.	0.001 ± 0.000^{B}	0.005 ± 0.001^{A}	0.019 ± 0.011^{B}	0.007 ± 0.002^{A}	0.003 ± 0.001^{B}	0.019 ± 0.014^{B}

Individual anthocyanins expressed in malvidin-3-glucoside equivalents (mg/g of berry)

Means of three replicates ± standard deviation

Different letters indicate the existence of statistical differences for each column ($p < 0.05$)

Delp gluc delphinidin-3-glucoside, *Cyan gluc* cyanidin-3-glucoside, *Petun gluc* petunidin-3-glucoside, *Peon gluc* peonidin-3-glucoside, *Malv gluc* malvidin-3-glucoside, *Cyan acet-gluc* cyanidin-3 acetylglucoside, *Petun acet-gluc* petunidin-3-acetylglucoside, *Peon acet-gluc* peonidin-3-acetylglucoside, *Malv acet-gluc* malvidin-3-acetylglucoside, *Peon coum-gluc* peonidin-3-*p*-coumaroyl glucoside, *Malv coum-gluc* malvidin-3-*p*-coumaroyl glucoside, *n.d.* not detected

a great number of grape varieties studied from both wine regions considered.

Cyanidin derivatives were one of the individual anthocyanin groups with the lowest concentration (cyanidin-3-glucoside and cyanidin-3-acetylglucoside) which is in agreement with the results published by several authors [41, 42]. According to Brar et al. [43], at beginning of ripening, the concentrations of peonidin-3-glucoside and cyanidin-3-glucoside in the berry skin are higher to compare to that of the other anthocyanins, including malvidin-3-glucoside. As the ripening progressed, the concentration of malvidin-3-glucoside increased and became similar or even higher than cyanidin-3-glucoside. Probably, there is a partial blockage of the anthocyanin biosynthetic pathway leading to the formation of trisubstituted anthocyanins as a result of different enzymatic activity. With the advancement of ripening, the activity of enzymes may have increased resulting in higher concentration of trisubstituted anthocyanins especially malvidin-3-glucoside as compared to cyanidin-3-glucoside. Several authors reported a high specific activity of 3′,5′-methyltransferase for the most labile anthocyanin substrate, delphinidin-3-glucoside, which is converted by this enzyme in two subsequent methylation steps to malvidin-3-glucoside [44, 45].

Antioxidant capacity and radical scavenger activity

The data in Fig. 4 show the antioxidant capacity and radical scavenger activity quantified in the different red grape varieties studied. Taking into account the global average values, the antioxidant capacity was significantly higher in the grape varieties from ‘Dão’ region vineyard (global average value of 6.57 and 5.92 μmol trolox/g of berry, respectively, for ABTS and DPPH methods) than from the same grape varieties from ‘Douro’ region vineyard (global average value of 3.93 and 4.21 μmol trolox/g of berry, respectively, for ABTS and DPPH methods). These results suggested that the antioxidant capacity of the Portuguese red grape varieties studied are also strongly affected by ‘terroir’ factors which confirm the results obtained for the general phenolic composition, where the red grape cultivars from the vineyards located in the ‘Dão’ region showed the highest total phenolic compounds, total anthocyanins and flavonoid compounds.

Correlations between antioxidant activity and total polyphenolic content of a great number of grape seed and skin extracts from different grape varieties have been reported by several authors [15]. Nevertheless, other authors [13] reported no significant correlations between individual flavanols analyzed by HPLC or total polyphenols and antioxidant capacity from several grape varieties. Recently, Jordão et al. [46] reported a linear negative correlation values between individual anthocyanins and antioxidant capacity during grape maturation, while a positive relationship between the different proanthocyanidin fractions and antioxidant capacity during grape maturation was also recently reported in other work [47]. Thus, there are conflicting evidence on literature about the correlation between polyphenol content and the antioxidant activity of grapes.

Considering the results for antioxidant capacity of the individual red grape varieties analyzed, a large variation was found. As seen in Fig. 4, the grape samples from ‘Dão’ region vineyard showed an average value for the antioxidant capacity that ranged from 5.12 to 9.30 μmol trolox/g of berry and from 2.93 to 9.52 μmol trolox/g of berry, respectively, considering the results obtained by the use of ABTS and DPPH method. For grape variety samples from ‘Douro’ region vineyard, the values for the antioxidant capacity ranged from 0.73 to 11.63 μmol trolox/g of berry and from 0.41 to 13.72 μmol trolox/g of berry, respectively, considering the results obtained by the use of ABTS and DPPH method. Recently, Costa et al. [4] evaluated the antioxidant activity in the different grape berry fractions (skins, pulps and seeds) from different red grape varieties cultivated in Portugal (included Portuguese and French grape varieties) and concluded that it was not possible to establish a clear difference among the grape varieties analyzed.

In general, all grape varieties harvested in ‘Dão’ region vineyard showed significantly higher antioxidant capacity values than the same grape varieties harvested in ‘Douro’ region vineyard. However, ‘Tinta Roriz’ grape variety was an exception because the sample harvested in the vineyard of ‘Douro’ region showed significantly higher antioxidant capacity values, independent of the antioxidant capacity methodology used (for example, for ABTS method, 11.63 and 9.30 μmol trolox/g of berry, respectively, for the samples harvested in the vineyard located in ‘Douro’ and ‘Dão’ regions). ‘Mourisco Tinto’ (0.41 and 0.73 μmol trolox/g of berry, respectively, for DPPH and ABTS methods) and ‘Touriga Fêmea’ (2.93 and 3.92 μmol trolox/g of berry, respectively, for DPPH and ABTS methods) showed the lowest antioxidant capacity values, respectively, for grape varieties from ‘Douro’ and ‘Dão’ regions vineyards.

The hydroxyl radical- and superoxide radical-scavenging activities of all grape varieties cultivated in the two wine regions considered are also shown in Fig. 4. Values of superoxide radical scavenger activity (SRSA) showed similar tendency in close agreement with those described by general phenolic parameters (except for non-flavonoid compounds) and antioxidant capacity. Thus, grape samples harvested in the vineyard located in ‘Dão’ region showed a significantly higher SRSA overall average value (47.5 %) than grape samples harvested in the vineyard located in ‘Douro’ region (38 %). On the other hand, grape samples harvested in the vineyard localized in ‘Douro’ region showed significantly higher hydroxyl radical scavenger

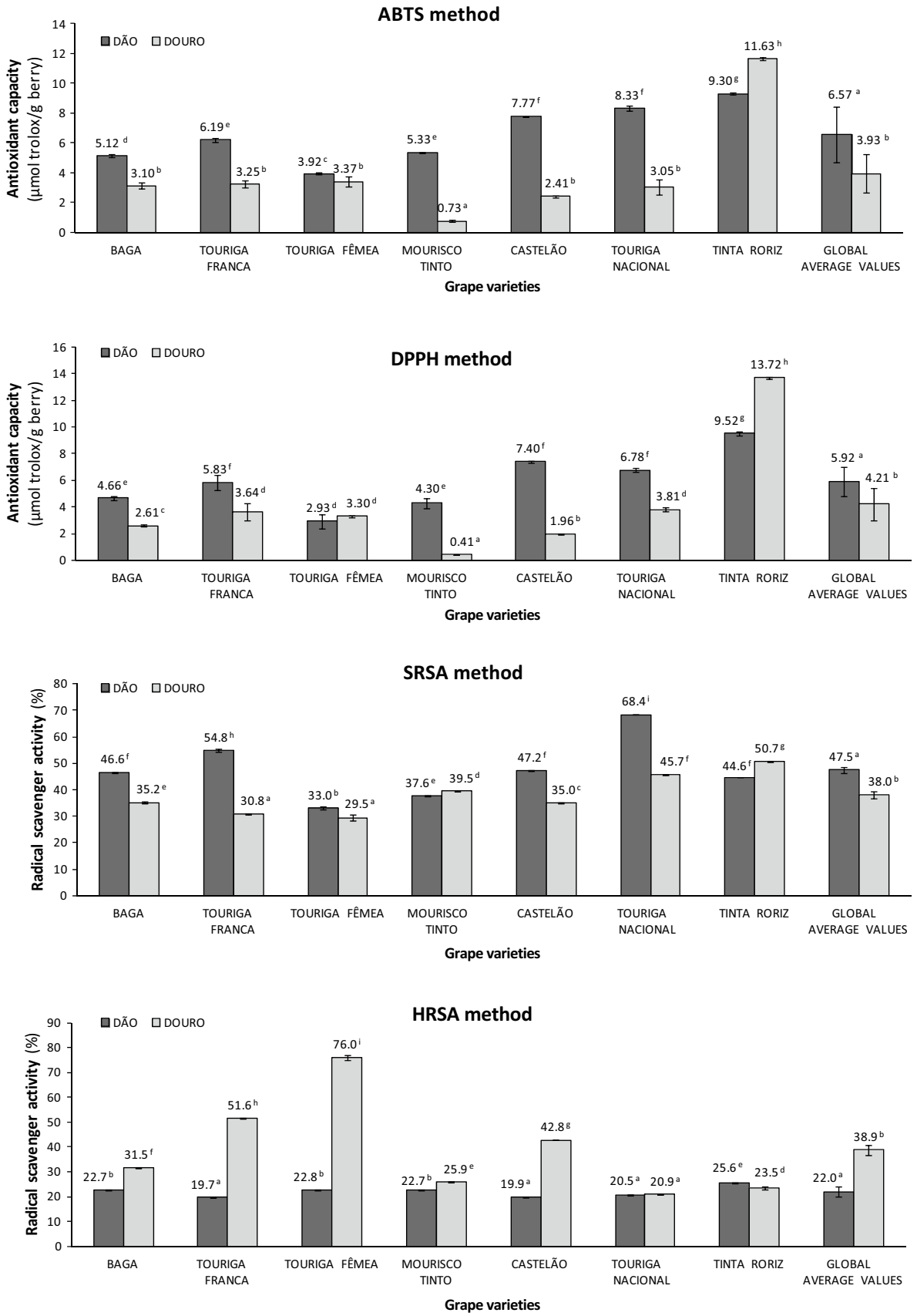

Fig. 4 Antioxidant capacity (ABTS and DPPH methods) and radical scavenger activity (SRSA and HRSA methods) from the grape varieties studied at technological maturity

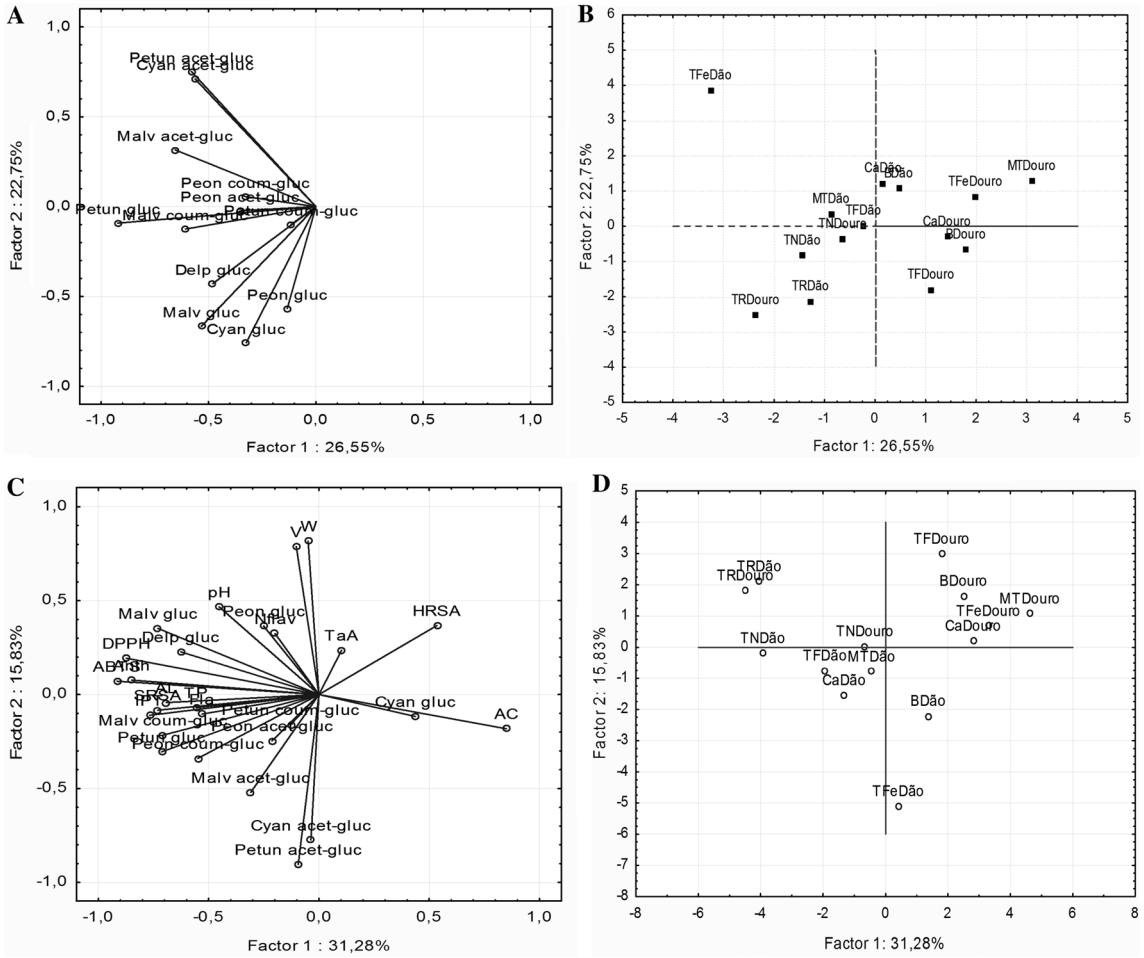

Fig. 5 Projection of principal component analysis data from the grape varieties studied when individual anthocyanin data were used (**a**, **b**) and when all data analyzed were integrated (**c**, **d**). *TFe* Touriga Fêmea, *B* Baga, *MT* Mourisco Tinto, *Ca* Castelão, *TF* Touriga Franca, *TR* Tinta Roriz, *TN* Touriga Nacional, *Delp gluc* delphinidin-3-glucoside, *Cyan gluc*, cyanidin-3-glucoside, *Petun gluc* petunidin-3-glucoside, *Peon gluc* peonidin-3-glucoside, *Malv gluc* malvidin-3-glucoside, *Cyan acet-gluc* cyanidin-3-acetylglucoside, *Petun acet-gluc* petunidin-3-acetylglucoside, *Peon acet-gluc* peonidin-3-acetylglucoside, *Malv acet-gluc* malvidin-3-acetylglucoside, *Peon coum-gluc* peonidin-3-*p*-coumaroyl glucoside, *Malv coum-gluc* malvidin-3-*p*-coumaroyl glucoside, *W* grape berry weight, *V* must volume, *Al* estimated alcohol degree, *Ac* titratable acidity, *TaA* tartaric acid, *IPT* total polyphenol index, *Anth* total anthocyanins, *TP* total phenolic compounds, *Flav* flavonoid compounds, *NFlav* non-flavonoid compounds

activity (HRSA) global average value (38.9 %) than the grape samples harvested in the vineyard localized in 'Dão' region (22.0 %).

Taking into consideration the individual grape varieties analyzed, 'Touriga Fêmea' and 'Touriga Franca' grapes from vineyard localized in the 'Douro' region showed the significantly highest HRSA values (76.0 and 51.6 %, respectively) while 'Castelão' and 'Touriga Franca' grape varieties harvested in 'Dão' region vineyard showed the lowest HRSA values (19.9 and 19.7 %, respectively). Finally, the highest SRSA values were found in 'Touriga Nacional,' 'Touriga Franca,' 'Castelão,' and 'Baga' grape varieties (68.4, 54.8, 47.2, and 46.6 %, respectively) all collected in the vineyard located in the 'Dão' region.

Principal component analysis

For better understanding the relationship between grape varieties, vineyard localization and individual anthocyanin composition, principal component analysis was performed. The data are shown in Fig. 5a, b. The two first PCs explained 49.3 % of the total variance. The first principal component (PC1—26.55 %) is negatively correlated with the variables petunidin-3-acetylglucoside, cyanidin-3-acetylglucoside, malvidin-3-acetylglucoside, peonidin-3-*p*-coumaroyl glucoside and peonidin-3-acetylglucoside and the second principal component (PC2—22.75 %) is negatively correlated with the variables petunidin-3-*p*-coumaroyl glucoside, petunidin-3-glucoside, malvidin-3-*p*-coumaroyl glucoside, delphinidin-3-glucoside, peonidin-3-glucoside,

malvidin-3-glucoside and cyanidin-3-glucoside (Fig. 5a). The grape varieties of 'Touriga Fêmea,' 'Castelão,' 'Baga' and 'Mourisco Tinto' are rather grouped on the positive side of PC2 due to their composition in acetyl glucoside groups and 'Touriga Franca,' 'Tinta Roriz' and 'Touriga Nacional' grape varieties on the negative side of PC2 due to their composition in glucoside groups (Fig. 5b).

Principal component analysis was also applied to all parameters analyzed in order to know the relationship between grape varieties, vineyard localization and the parameters analyzed in the red grape varieties studied. The correlation score plot in Fig. 5c, d showed that the first principal components (PC1) accounted for 31.28 % of the variability and the second principal component (PC2) accounted for 15.83 % of the variability and together PC1 and PC2 accounted for 47.11 % of the total variance. Using the plots in Fig. 5c, d, it is also possible to locate the grape varieties on basis of their chemical composition, and consequently, they could be separated according to their origin ('Dão' or 'Douro'). The grape varieties from the vineyard localized in the 'Douro' region are rather grouped on the positive side of PC2 due to their composition of non-flavonoid compounds, monoglucoside anthocyanins, antioxidant capacity and hydroxyl radical scavenger activity (HRSA method), and the grape varieties from the vineyard localized in the 'Dão' region are grouped on the negative side of PC2 due to their composition of acetyl and coumaryl glucoside groups, total phenols, flavonoids and superoxide radical scavenger activity (SRSA method).

Conclusions

In general (except for 'Touriga Nacional' and 'Tinta Roriz'), red grape varieties cultivated in vineyard from 'Dão' wine region have higher phenolic content (total phenolic compounds, total and individual anthocyanins and flavonoid compounds) than the same red grape varieties cultivated in a vineyard from 'Douro' region, except for non-flavonoid compounds. Same tendency was obtained for the total antioxidant capacity measured by the two methodologies used (ABTS and DPPH methods).

Regarding the scavenger activity, the superoxide radical scavenger activity (SRSA) values showed similar tendency in close agreement with those describe by general phenolic parameters and total antioxidant capacity while hydroxyl radical scavenger activity (HRSA) values showed an opposite tendency probably as a consequence of higher non-flavonoid content quantified in red grape samples from the vineyard localized in 'Douro' region. In relation to the individual red grape varieties studied, 'Touriga Nacional' and 'Tinta Roriz' showed in general the highest phenolic content and total antioxidant capacity, especially in 'Douro' region. Thus, probably these two grape varieties are the most adapted to the conditions of 'Dão' and 'Douro' wine regions.

Finally, as a final consideration, it is important to note that the adaptation degree of certain grape varieties to the climate and soil conditions may be a differentiating factor with respect to the accumulation and extractability of phenolic compounds in the grapes and in their antioxidant capacity. However, the results obtained in our work need to be treated with caution, because variables such as vintage or harvest date could influence the relative values of the parameters analyzed.

Conflict of interest None.

Compliance with Ethics Requirements This article does not contain any studies with human or animal subjects.

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